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Morphology, molecular phylogeny, and taxonomic inconsistencies in the study of *Bradypus* sloths (Pilosa: Bradypodidae)

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This study focuses on morphological and molecular data analyses, misidentifications, and phylogenetic inconsistencies regarding *Bradypus variegatus* (the brown-throated sloth) and *B. tridactylus* (the pale-throated sloth). Misidentifications were recorded on 75 of 313 museum specimens of *Bradypus*. Almost 90% of the misidentified specimens were *B. variegatus* from north-central Brazil, erroneously attributed to *B. tridactylus*. These misidentified specimens are reported in taxonomic reviews as the southernmost records of *B. tridactylus*. A history of confusing nomenclature regarding sloth species exists, and these particular misidentifications could be attributable to the similarity in face and throat color between *B. variegatus* from north-central Brazil and *B. tridactylus.* The molecular phylogeny of morphologically confirmed sloth specimens exhibits 2 monophyletic lineages representing *B. variegatus* and *B. tridactylus*. The split time between these 2 lineages was estimated at 6 million years ago (mya), contradicting previous studies that estimated this divergence to be 0.4 mya. Taxonomic inconsistencies were detected when comparing the molecular phylogeny to previously published DNA sequences ascribed to *B. tridactylus.* Misidentification or introgression could underlie such phylogenetic incongruities. Regardless of their causes, these discrepancies lead to misstatements regarding geographic distribution, phylogeny, and taxonomy of *B. variegatus* and *B. tridactylus*.

Key words: misidentification, molecular data, phylogenetic incongruity, three-toed sloth

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The development of molecular markers and increasing knowledge about the processes and rates of molecular change have provided remarkable tools to detect, describe, and explain biological diversity. Therefore, an ever-growing resource of DNA sequences has become available, mostly over the Internet as public databases (such as GenBank). These databases represent an important resource for studies of phylogeny, biogeography, phylogeography, conservation genetics, molecular taxonomy, genetic diversity, and intraspecific units as targets for conservation (Nikolaev et al. 2007; Ranwez et al. 2007; Thomas 2008).

Beyond the basic utility and value of such databases, several issues regarding the use of these DNA data in making evolutionary, phylogenetic, or biodiversity inferences are important. Usually, molecular phylogenetic studies are concerned with the characteristics of genes and the methods used to construct trees. These aspects include homoplasy, introgression, deviation from neutrality, rate heterogeneity among taxa, confidence in estimated molecular trees, and artifacts such as long-branch attraction. However, elementary attributes such as the correct identification of specimens also warrant careful consideration (Hawksworth 2004; Vilgalys 2003). The most frequent taxonomic errors occur in those groups of organisms where identification is particularly challenging, because of the small size of the species involved, morphological similarity among them, or lack of adequate tools for identification. These issues are common for species of fungi, insects, nematodes, and protists. However, the problem applies across a wide spectrum, from cell lines to large mammals (Hawksworth 2004).

A case of taxonomic misidentification cited in the literature relates to 2 Neotropical mammals, the brown-throated sloth (*Bradypus variegatus*) and the pale-throated sloth (*B. tridactylus*). *B. variegatus* is a widely distributed species occurring throughout most of Central and South America. It is sympatric with *B. tridactylus* in northern Brazil along the

Negro and Amazon rivers. *B. tridactylus* also is found in Guyana and adjacent regions of east-central Venezuela and north-central Brazil (Gardner 2007; Wetzel and Ávila-Pires 1980). *B. variegatus* and *B. tridactylus* are distinguishable by color differences in the hairs of the face and throat and by a pair of foramina at the anterodorsal nasopharynx present only in *B. tridactylus* (Wetzel and Ávila-Pires 1980). Although morphological differences occur between these sloths, in the older literature most *B. variegatus* were referred erroneously to *B. tridactylus* (Anderson and Handley 2001; Gilmore et al. 2000). In the review of *Bradypus* by Anderson and Handley (2001) the authors presented a list of analyzed museum specimens but did not indicate which specimens were misidentified.

Correct taxon assignment is of paramount importance to conservation of biodiversity because inappropriate decisions can be made if taxonomic assignments are incorrect. Within *Bradypus*, the maned sloth (*B. torquatus*) and the pygmy sloth (*B. pygmaeus*) are threatened with extinction because of their restricted geographical distributions and loss of habitat. *B. variegatus* and *B. tridactylus* are considered of least concern (International Union for the Conservation of Nature and Natural Resources 2009). However, few studies exist on natural populations, especially regarding demography, and such investigations could improve understanding of the endangerment status of species. Analyses of population genetics data have been reported for only 2 sloth species, *B. torquatus* and *B. variegatus* (Lara-Ruiz et al. 2008; Moraes-Barros et al. 2006, 2007). The remaining molecular data generated for three-toed sloths are mostly from studies describing the placement of Xenarthra in eutherian phylogeny (Arnason et al. 1997; Eizirik et al. 2001; Murphy et al. 2007; Prasad et al. 2008) or on sloth phylogeny (Barros et al. 2003, 2008; Greenwood et al. 2001; Poinar et al. 2003). In GenBank ~11% of the total nonprimate eutherian DNA data available (until February 2010) are from xenarthrans. Although the proportion of xenarthran DNA data is high and similar to that allotted to other mammalian groups such as carnivores (11%) and insectivores (7%), only 0.008% of the xenarthran DNA sequences are from *Bradypus*.

A better characterization of sloth diversity is needed, given the few molecular studies published to date and the possibility of misidentification between *B. variegatus* and *B. tridactylus*. Discussions on molecular systematics, evolution, and genetic diversity can result in incorrect conclusions when taxonomic identification is inaccurate. Here we describe the occurrence of misidentification related to the brown and pale-throated sloths, investigate incongruities in molecular phylogenies, and discuss the implications of these problems for current knowledge of three-toed sloths. We used comparative analysis of morphological and molecular data obtained from specimens sampled in nature, museum collections, and online DNA databases.

Fig. 1.—Geographic distributions of *Bradypus variegatus* and *B. tridactylus* according to the International Union for the Conservation of Nature and Natural Resources Global Mammal Assessment and Edentate Specialist Group (Aguiar 2004). The dashed line delimits the inferred region of sympatry. Symbols indicate localities of analyzed specimens for the 2 species (black circles = *B. variegatus*; open circles = *B. tridactylus*; open triangles = both species), identified according to morphological criteria.

Materials and Methods

Specimens and DNA sequences.—To review the taxonomic identity of three-toed sloths we examined the morphology of skulls from the following natural history museums: AMNH—American Museum of Natural History, New York; FMNH—Field Museum of Natural History, Chicago, Illinois; IEPA—Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá, Amapá, Brazil; MN—Museu Nacional, Rio de Janeiro, Rio de Janeiro, Brazil; MPEG—Museu Paraense Emílio Goeldi, Belém, Pará, Brazil; MZUSP—Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo, Brazil; and USNM—United States Natural History Museum, Washington, D.C. We performed molecular analysis, comparing DNA sequences obtained from taxonomically reviewed specimens to sequences available from GenBank. All information regarding specimens is described in Appendix I.

Taxonomic identification based on morphology.—Three-toed sloth museum specimens (Appendix I) had their species identity established by the presence or absence of the paired foramina in the anterodorsal nasopharynx (Anderson and Handley 2001; Wetzel and Ávila-Pires 1980). Because cranial morphology was used for taxonomic identification, specimens lacking skulls or with crania broken at the nasopharynx region were not included. Specimen identity was compared with the previously attributed taxon name and corrected as needed. For some museum specimens molecular data also were obtained (Moraes-Barros and Morgante 2007) and used in phylogenetic analysis. The main localities of analyzed specimens and the currently inferred geographic distributions of *B. variegatus* and *B. tridactylus* are shown in Fig. 1.

Molecular phylogeny and taxonomic inconsistencies.—We estimated molecular phylogenetic trees using segments of mitochondrial cytochrome-*b* (*Cytb*; 471 base pairs [bp]) and 16S rRNA (16S; 498 bp) genes obtained only from *Bradypus* sloths for which taxonomic identification could be confirmed by morphological analysis (DNA control data sets). The analyzed specimens encompassed *B. tridactylus*, *B. variegatus*, and *B. torquatus*. For *B. tridactylus* only 2 individuals from the same locality were analyzed. Specimens of *B. variegatus* included representatives of the *B. variegatus* Management Units described by Moraes-Barros et al. (2007). A total of 8 individuals representing the 6 distinct *B. variegatus* Management Units and 2 *B. torquatus* were analyzed (Appendix I). DNA samples were obtained from museum specimens (ethanol-preserved, frozen tissues, or study skins) and from living animals (blood samples). Living specimens were captured in nature (Appendix I) and identified from external morphology (Anderson and Handley 2001). Blood samples were collected for DNA analysis according to specific permits (Ibama 02001.000877/2003; Ibama/ICMBio 19267-1) and animal handling and care were consistent with guidelines of the American Society of Mammalogists (Gannon et al. 2007). After sampling, animals were released. Blood samples were transferred to tubes containing ethanol or heparin and stored at -20°C. These samples were deposited in our DNA and tissue collection (Laboratorio de Biologia Evolutiva e Conservação de Vertebrados [LABEC]; Appendix I). Methods used to extract, amplify, and sequence DNA varied according to the level of degradation and source of DNA (ethanol-preserved, frozen tissue, blood, or museum study skins) and were performed according to the protocol and primers in Moraes-Barros and Morgante (2007).

Phylogenetic relationships were reconstructed in PAUP* version 4.0b10 (Swofford 2002) through maximum-likelihood and neighbor-joining methods, depending on the analysis as discussed below. The maned sloth (*B. torquatus*), two-toed sloth (*Choloepus didactylus*), and the southern tamandua (*Tamandua tetradactyla*) were used as outgroups. The Akaike information criterion (AIC) implemented in the computer program Modeltest version 3.06 (Posada and Crandall 1998) was used to identify the most appropriate model of DNA substitution for each data set (sets of concatenated *Cytb* and 16S genes and independent data sets for each gene). The best model found for the data set of concatenated *Cytb* and 16S genes was the general time reversible (GTR) model (Rodríguez et al. 1990) including gamma distribution with shape parameter (α). The Tamura–Nei (TrN) model (Tamura and Nei 1993) including proportion of invariant sites (I) was identified as the best model for the independent data sets of *Cytb* and 16S. The analyses were carried out with a heuristic search using the tree-bisection-reconnection branch swapping algorithm and ''as is'' addition. The robustness of trees was determined by 1,000 (neighbor-joining) and 100 (maximum-likelihood) bootstrap replications.

Control data sets (each gene as an independent data set and a set of concatenated gene sequences) were 1st used to infer maximum-likelihood and neighbor-joining trees. We estimated divergence times for the main *Bradypus* lineages to compare our data with published studies on split times between *B. variegatus* and *B. tridactylus*. Barros et al. (2003, 2008) used mitochondrial 16S and 12S genes to estimate a split between *B. variegatus* and *B. tridactylus* of about 400,000 years ago. We used the maximum-likelihood phylogeny based on control data set of concatenated *Cytb* (471 bp) and 16S (498 bp) gene segments. Estimates of divergence times were obtained using the penalized likelihood method (Sanderson 2002) implemented with program R8s 1.7.1 (Sanderson 2003). Not having fossil records of arboreal sloths or the mitochondrial DNA (mtDNA) substitution rate for *Bradypus*, we used the same criteria of Barros et al. (2003, 2008) to calibrate the tree and estimate divergence times. Our calibration point was the split between *Choloepus* and *Bradypus*, which occurred 21–18 million years ago (mya—Delsuc et al. 2001, 2004).

We expected that molecular trees, obtained with the control data sets, would show distinct monophyletic groups corresponding to each sloth species. This phylogenetic pattern would indicate congruence between molecular phylogeny and taxonomy. Conversely, any observed incongruence would be evidence of incomplete lineage sorting or introgression. After this 1st analysis new phylogenies were obtained using the control data sets plus homologous DNA sequences identified as *B. variegatus* and *B. tridactylus* available in GenBank. Each gene was considered an independent data set so the distinct sequences available in GenBank could be evaluated. Phylogenies were estimated with the neighbor-joining method because of its computational speed and high accuracy, especially when the evolutionary dynamics among the sequences have remained the same over time. Therefore, in these analyses we considered only sequences of *B. variegatus* and *B. tridactylus*, using *B. torquatus* as outgroup. We assume that if the molecular phylogeny of morphologically reviewed specimens is congruent with taxonomy, phylogenetic inconsistencies involving DNA sequences from GenBank could be explained by misidentification. We could not analyze all DNA sequences of *B. variegatus* and *B. tridactylus* available in GenBank because our control data set was limited to only 2 genes. Therefore, we investigated only *Cytb* and 16S sequences. DNA sequences generated in this study are available in GenBank under accession numbers HM352889–HM352908.

Results

Taxonomic attribution based on cranial morphology.—We identified 313 sloth museum specimens based on cranial morphology without difficulty, except when crania were broken at the anterodorsal nasopharynx, as for specimen MZUSP 23159. For this particular specimen analysis of hairs on the face and throat on the preserved skin was possible, and we obtained DNA for molecular analysis. We found misidentification events in almost all collections except the
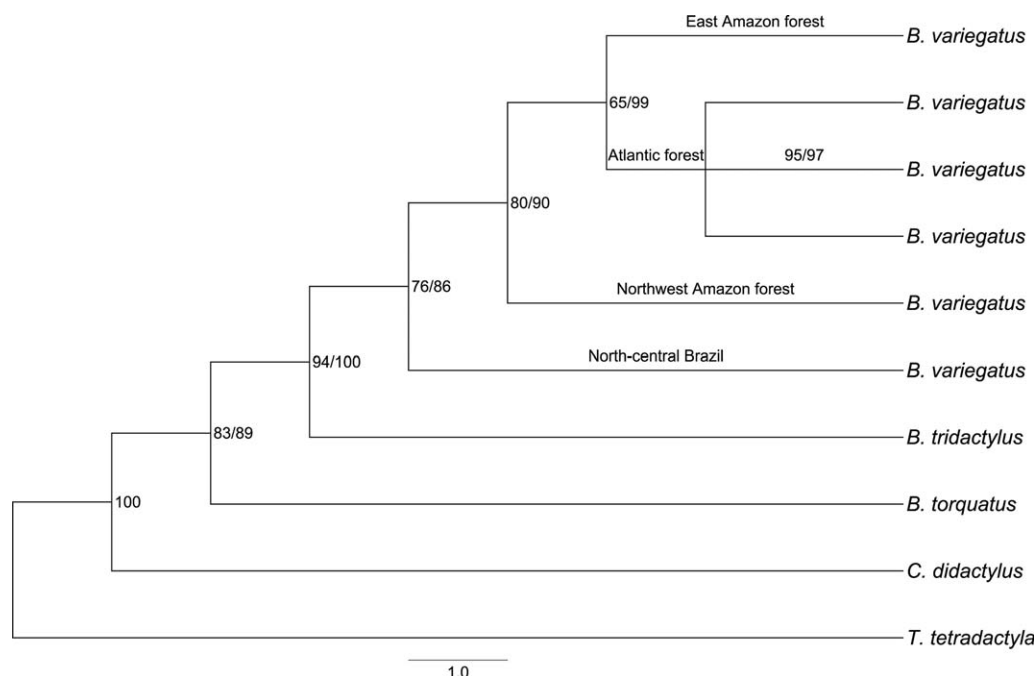
Fig. 2.—Bootstrap maximum-likelihood (ML) consensus tree obtained using concatenated cytochrome-*b* (*Cytb*) and 16S sequences from morphologically validated specimens of *Bradypus*. Tree was estimated using the general time reversible model (Rodríguez et al. 1990) and gamma distribution with shape parameter $\alpha = 0.3089$. Similar topologies were obtained in neighbor-joining (NJ) trees. Numbers at the nodes indicate maximum-likelihood–neighbor-joining bootstraps. Terminals for *B. variegatus* are identified according to the geographic location of sampled individuals and reflect exclusive mitochondrial DNA lineages previously described in Moraes-Barros et al. (2007). Genbank DNA sequences from *Choloepus didactylus* (accession number Z48942) and *Tamandua tetradactyla* (accession number NC004032) were used as outgroups. Scale at the bottom represents number of substitutions per site.

USNM collection. About 24% of all specimens (75/313) were misidentified, and 96% of the misidentifications were individuals of *B. variegatus* erroneously assigned to *B. tridactylus.* Also, 65 of the 75 misidentified specimens were from north-central Brazil (Appendix I).

Molecular phylogeny, taxonomic incongruity, and divergence times.—Using only DNA sequences from specimens whose taxonomic identification was confirmed, the *Cytb* and 16S data sets revealed similar topologies. Thus, we used the concatenated sequences for analysis considering the GTR model and $\alpha = 0.3089$. The resultant data set was 969 bp and recovered 2 main clades corresponding to *Bradypus* species. Within *B. variegatus* distinct clades corresponding to Management Units previously described by Moraes-Barros et al. (2007; Fig. 2) were observed.

We obtained a *Cytb* sequence from 1 specimen (MZUSP 23159) for which taxonomic identification could only be inferred based on the hair colors of the face and throat. The color pattern was characteristic of *B. tridactylus*, and the DNA sequence was similar to sequences obtained from 2 morphologically identified members of the same species (Fig. 3).

Phylogenies of *Cytb* and the 16S mtDNA sequences available in GenBank were estimated using the TrN model including $I = 0.6928$ (*Cytb*) and $I = 0.6363$ (16S). We observed inconsistencies in DNA sequences between the attributed name and the molecular phylogeny. GenBank sequences, putatively derived from either *B. variegatus* or *B tridactylus*, grouped together in a clade representative of *B. variegatus* (Figs. 3 and 4).

According to our results, the time of split between *B. tridactylus* and *B. variegatus* occurred between 6.0 and 4.8 mya, depending on the maximum and minimum values of the calibration point (21–18 mya). We also calculated divergence times for the split between *B. torquatus* and the *B. variegatus*–*B. tridactylus* lineages (14–11 mya) and the base of diversification of all *B. variegatus* lineages (5.0–3.8 mya).

Discussion

Despite having no difficulty identifying sloth specimens based on cranial morphology, we observed numerous taxonomic misidentifications, as previously reported in the literature. These misidentified specimens were detected in all museum collections (AMNH, FMNH, MN, MPEG, and MZUSP) except USNM. Although misidentifications occurred in the official collection lists from most museums, corrections to the original identification were attached to the specimens from AMNH, FMNH, and MPEG. Therefore, the use of sloth museum collection lists, without careful examination of specimens, can be problematic.

Bradypus variegatus and *B. tridactylus* have a confusing nomenclatural history. A complete and recent description of this topic is presented in Hayssen (2009, 2010). In short, after Schinz (1825) described *B. variegatus*, more than 50 names were applied to the species, including ''*B. tridactylus*'' (De Blainville 1840; Trouessart 1898; Vieira 1955). Throughout this period ''*B. tridactylus*'' was what is now *B. variegatus*,
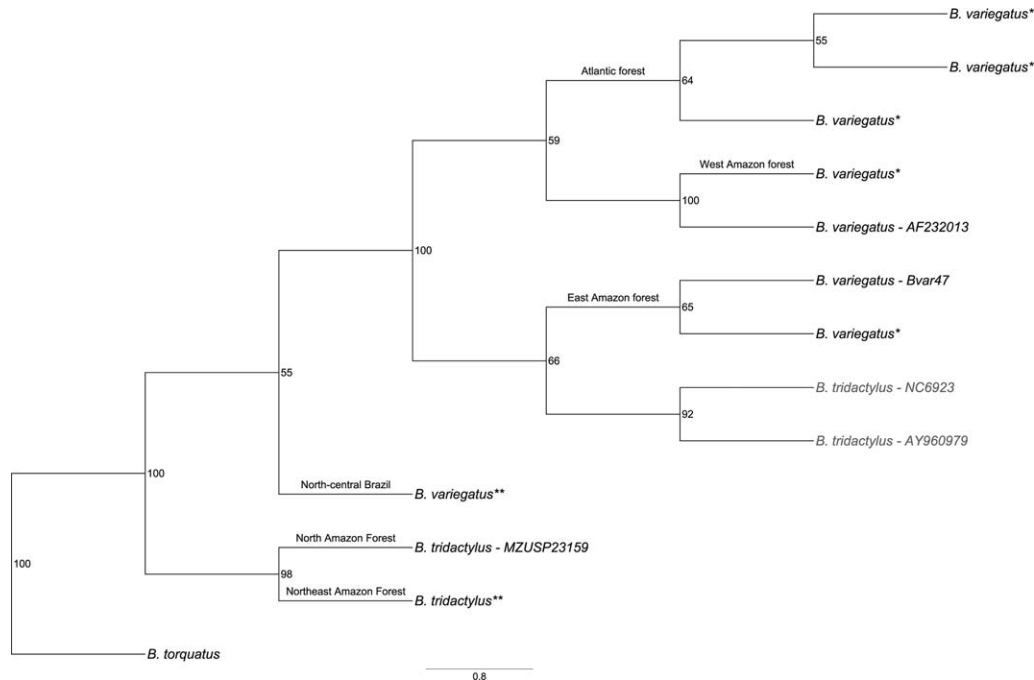
Fig. 3.—Neighbor-joining (NJ) phylogeny of cytochrome-*b* sequences of *Bradypus* from GenBank (identified by accession numbers) and from specimens identified based on external (*) and cranial (**) morphology. Tree was inferred using the Tamura–Nei model (Tamura and Nei 1993) with a proportion of invariant sites I = 0.6928. Numbers at the nodes indicate bootstrap values from 1,000 replications. Terminals in light gray indicate phylogenetic incongruities. Scale at the bottom represents number of substitutions per site.
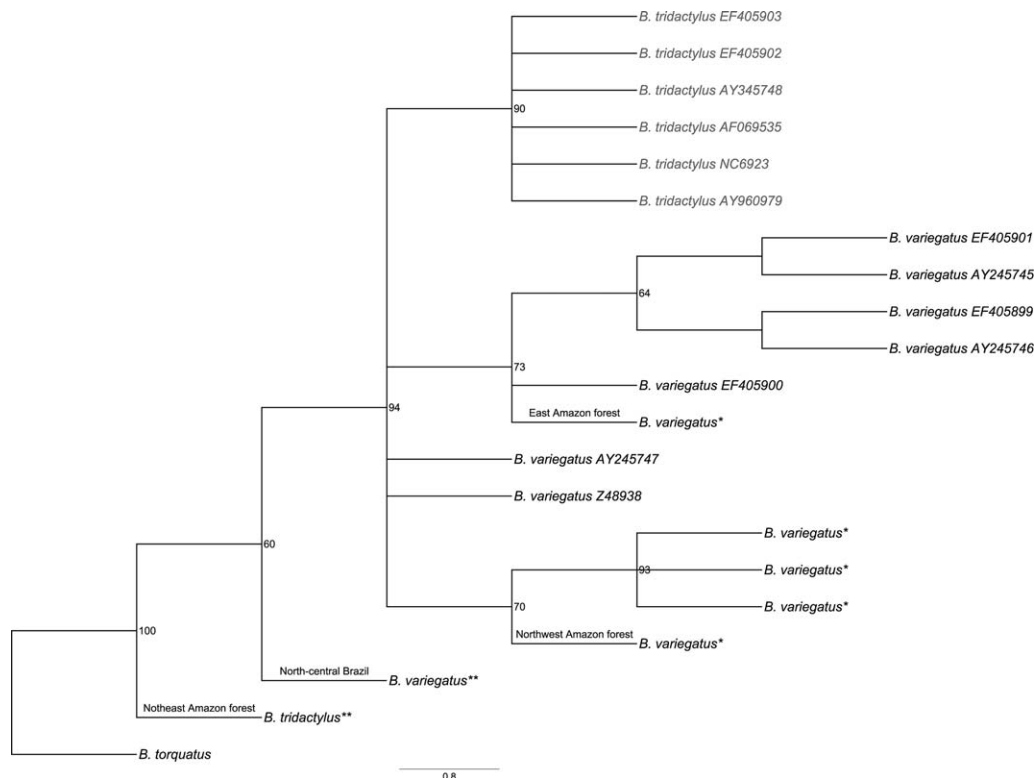
Fig. 4.—Neighbor-joining (NJ) phylogeny of 16S sequences of *Bradypus* from GenBank (identified by accession numbers) and from specimens identified based on external (*) and cranial (**) morphology. Tree was inferred using the Tamura–Nei model (Tamura and Nei 1993) with a proportion of invariant sites I = 0.6363. Numbers at the nodes indicate bootstrap values from 1,000 replications. Terminals in light gray indicate phylogenetic incongruities. Scale at the bottom represents number of substitutions per site.

not as a result of misidentification but in agreement with the accepted nomenclature. For instance, Ávila-Pires and Gouveia (1977) described a brown-throated sloth from an Atlantic forest nature reserve as ''*B. tridactylus*.'' Wetzel (1982) clarified the nomenclature of the 2 species, but problems persist in correctly assigning species names to individual specimens. The morphology of some *B. variegatus* populations, as we discuss below, contributes to this difficulty.

We observed that 96% of the misidentified specimens were *B. variegatus* erroneously attributed to *B. tridactylus*. Also, the majority of misidentified *B. variegatus* were from north-central Brazil where the 2 species might be sympatric. A history of confusing nomenclature exists, and these particular misidentifications could be attributable to the similarity in face and throat color between the north-central Brazil *B. variegatus* and *B. tridactylus.* Most specimens of the two species show pronounced differences in hair colors of the face and throat. *B. tridactylus* has bright golden-yellow hairs, whereas *B. variegatus* has a brownish face and throat, at least at the base of the hairs. In addition, most individuals of *B. variegatus* have a dark facial stripe not present in *B. tridactylus.* However, a few populations of *B. variegatus* from north-central Brazil (e.g., on the lower Tapajós River) exhibit a strong golden frosting on the throat. Unlike *B. tridactylus*, which has golden color to the base of the hairs, the hairs on *B. variegatus* are usually dark brown at the base (Anderson and Handley 2001). Therefore, misidentification of *Bradypus* sloths in museum collections also can arise from incomplete analysis of morphological traits.

Despite the apparent similarities of facial hair color among some populations of *B. variegatus* and *B. tridactylus*, cranial morphology and molecular data are diagnostic. Museum specimens of *B. variegatus* from north-central Brazil, with face and throat pelage similar to that of *B. tridactylus*, did not have a pair of nasopharyngeal foramina, a trait exclusive to *B. tridactylus.* Also, mtDNA sequences obtained from 1 of these specimens did not group with homologous sequences of *B. tridactylus*.

The molecular phylogeny based only on DNA sequences of morphologically reviewed specimens showed no incongruity. We then added to the analysis DNA sequences from specimens whose identification could not be confirmed by morphological analysis. One of these was museum specimen MZUSP 23159. This sloth was originally attributed to *B. tridactylus*, and its identification was confirmed based only on analysis of face and throat hairs. The obtained DNA sequence revealed a haplotype similar to that observed in 2 specimens of *B. tridactylus* identified based on cranial and pelage traits. Therefore, we assume that specimen MZUSP 23159 is *B. tridactylus.*

The mtDNA sequences from GenBank had inconsistencies between attributed species names and the molecular phylogeny. All GenBank sequences putatively derived from either *B. variegatus* or *B. tridactylus* grouped with *B. variegatus.* Therefore, the inconsistencies recorded were from GenBank DNA sequences erroneously attributed to *B. tridactylus*.

Incongruity in the topology of molecular phylogenies can be caused by incomplete lineage sorting, introgression, or taxonomic misidentification (Avise 2004). The process of incomplete lineage sorting is more likely to occur in sister species shortly after their separation or at intermediate times since speciation (Avise 2004). Our molecular dating indicates the split between *B. variegatus* and *B. tridactylus* to be 6.0–4.8 mya. With a 6-year generation time and a life span of 30–40 years for three-toed sloths (Anderson and Handley 2001), *B. variegatus* and *B. tridactylus* sharing ancient mtDNA haplotypes would be unlikely.

The occurrence of ancient hybridization and backcrosses between these 2 species could have led to introgression of mtDNA, resulting in individuals morphologically matching 1 species but carrying mtDNA of the other. Mitochondrial DNA introgression is only detectable using multiple markers, including morphology and molecular data or distinct genes from mitochondrial and nuclear genomes (Good et al. 2008). We did not observe incongruity comparing morphological and molecular data, which could have been evidence of introgression. Our molecular phylogeny included sloths from Atlantic and Amazon forests and encompassed most of the region where *B. variegatus* and *B. tridactylus* might be sympatric. All DNA sequences were obtained from individuals identified based on external or skull morphology. The corresponding DNA sequences presented a phylogenetic pattern consistent with the taxonomic identifications, and all mtDNA lineages of *B. variegatus* coalesce after the split between the brown-throated and pale-throated three-toed sloths. However, although our control data sets are constituted from DNA sequences obtained from sloths from different geographical regions, we did not sample most of the geographical distribution of *B. tridactylus*. We have sampled only 2 individuals of *B. tridactylus* that share the same haplotype. Thus, we can discard introgression from *B. tridactylus* into *B. variegatus* only for our control data sets. Nevertheless, phylogenetic discrepancies in GenBank DNA sequences of *Bradypus* also could have occurred due to misidentifications. However, no information exists as to the source of those DNA sequences in the Genbank files, so we cannot confirm incorrect identification or discard introgression as the cause of this phylogenetic mismatch. In molecular phylogenetic studies in cuckoos and Old World finches, Sorenson and Payne (2001, 2002) and Payne and Sorenson (2003) were able to confirm the identification of 120 specimens and correct it for another 8. However, this was possible only by comparing the genetic data to specimen vouchers.

Correctness in taxonomy is important when analyzing DNA samples. When inconsistencies in molecular phylogeny are detected, it is impossible to investigate the cause of incongruity when no associated preserved specimen is available. However, for some species (such as those threatened with extinction), preserving each specimen from which DNA was obtained is not realistic. In these cases, DNA vouchers associated with published sequences would be recommended.

If phylogenetic inconsistencies are detected in a published molecular data, posterior analysis of different and independent molecular markers can made with the associated DNA.

Whatever the causes of inconsistencies in molecular phylogenies, the Genbank DNA sequences of *Bradypus* clearly are mismatched. Our aim is not to denigrate previous studies or public DNA databases but rather to raise awareness among investigators who use these data in molecular biology studies. The dissimilar DNA sequences of *B. tridactylus* encompass a complete mitochondrial genome analysis (*Cytb* and 16S—McLenachan and Penny 2005), sequences used in a phylogenetic study of the relationships among the main mammalian orders (16S—Stanhope et al. 1998), and DNA segments used to study the phylogeny of sloth species (16S—Barros et al. 2003). The observed divergences do not impose errors in the phylogenetic discussion of Stanhope et al. (1998), because the DNA sequence was used only to represent a three-toed sloth, which is correct. However, the assumption that any of these DNA sequences represent an mtDNA lineage of *B. tridactylus* is mistaken.

According to our analysis of the control data set of concatenated *Cytb* and 16S, the split between mtDNA lineages of *B. torquatus* and those of the remaining *Bradypus* occurred about 12 mya, a date corresponding to that obtained by Barros et al. (2003, 2008). The inferred split time between *B. tridactylus* and *B. variegatus* was between 6.6 and 4.8 mya, depending on the calibration point (21–18 mya). This date differs from the 0.4 mya estimated by Barros et al. (2003, 2008) using 16S and 12S mtDNA sequences. Considering the results presented here, we assume that the date obtained by Barros et al. (2003, 2008) applies to the divergence between 2 mitochondrial lineages of *B. variegatus*. The split between *B. variegatus* and *B. tridactylus*, estimated in our analysis at about 6 mya, could have been missed if a wide sampling of taxonomically reviewed specimens had not been included in the analysis. This inferred date agrees with Delsuc et al. (2002), who argued that most xenarthran diversification occurred in the Miocene or early Pliocene.

In the case of three-toed sloths, phylogenetic inconsistencies and missing data seem to have influenced previous studies on sloth evolutionary history. Although we cannot state that misidentifications underlie the taxonomic incompatibility observed for available GenBank DNA sequences, our morphological analyses of museum specimens of *Bradypus* prove that taxonomic misidentifications do occur, as previously suggested by Anderson and Handley (2001). A critical outcome of the misidentifications is the inferred geographical distribution of *B. variegatus* and *B. tridactylus*. The actual distribution of *B. tridactylus* is narrower than previously inferred (Gardner 2007; International Union for the Conservation of Nature and Natural Resources 2009). Some misidentified *B. variegatus* were reported in taxonomic reviews as records of *B. tridactylus*. These specimens are *B. variegatus* from Colombia and north-central Brazil, southward to the Amazon River (Fig. 1). We agree with Anderson and Handley (2001), who pointed out that the distribution of *B. tridactylus* probably does not extend southwest of the Rio Negro or as far south of the Amazonas River. We do not agree with Hayssen (2010), who suggested that *B. variegatus* does not occur north of the Amazon. There are morphologically confirmed *B. variegatus* from north of the Amazon in the MUZUSP and MPEG collections (Appendix I). Therefore, the geographic distributions of *B. variegatus* and *B. tridactylus* need further clarification.

A better understanding of three-toed sloth genetic diversity also is needed. This is particularly important if we consider the genetically divergent populations within the species, as described in recent phylogeography studies (Moraes-Barros et al. 2006, 2007). These authors identified genetically distinct populations of *B. variegatus* distributed throughout the Amazon and Atlantic forest. These populations were classified as Management Units and indicated as intraspecific targets for conservation purposes. The mtDNA lineages that constitute these Management Units were inferred from segments of the control region, a highly polymorphic DNA segment. Our phylogenetic analysis corroborates the divergence among these lineages. Moreover, additional mtDNA lineages of *B. variegatus* were revealed by adding GenBank sequences to the phylogenies. Most of the GenBank DNA sequences that group with *B. variegatus* are identified as *B. tridactylus*. These divergent sequences represent about 20% of the observed mtDNA diversity of *B. variegatus*. This indicates that genetic diversity within *B. variegatus* is higher than previously reported. Also, marked divergences occur among haplogroups of *B. variegatus*, suggesting the existence of different and independent evolutionary lineages. For example, the mtDNA lineage representing sloths from north-central Brazil is divergent and basal within the *B. variegatus* group. In our study only the South American *B. variegatus* and 3 *B. tridactylus* from northern Brazil were analyzed. *B. variegatus* also is distributed throughout Central America, and no study has been performed on the molecular diversity of *B. tridactylus* from the Guiana Shield. Therefore, to investigate potential speciation events properly and confirm the reciprocal monophyly of *B. variegatus* and *B. tridactylus*, wider geographic sampling and the use of independent molecular markers will be needed. Nevertheless, our results show the importance of accurate specimen identification in molecular systematics.

Resumo

Este trabalho tem como foco a análise de dados morfológicos e moleculares, o estudo de erros de identificação e de inconsistências filogenéticas, referentes às espécies *Bradypus variegatus* (preguiça de garganta marrom) e *B. tridactylus* (preguiça de garganta clara). Foram registrados erros de identificação em 75 dos 313 espécimes analisados em diferentes coleções científicas. Cerca de 90% dos erros de identificação foram observados em *B. variegatus*, provenientes da região centro norte do Brasil, erroneamente identificados como *B. tridactylus*. Esses espécimes são citados

na literatura como registros do limite sul da distribuição de *B. tridactylus*. A história da nomenclatura destas espécies de preguiça denota certa confusão. Ainda assim, os erros de identificação destes espécimes em particular pode ser atribuído às semelhanças na coloração dos pelos da face e garganta, observadas entre *B. variegatus* da região centro norte do Brasil e *B. tridactylus*. A filogenia molecular de espécimes de preguiça, cuja identificação foi confirmada através de dados morfológicos, denota 2 grupos monofiléticos representantes das espécies *B. variegatus* e *B. tridactylus*. A divergência entre estes 2 grupos foi datada em 6,0 ma. Este resultado contradiz estudos anteriores os quais estimaram esta divergência em 0,4 ma. Ademais, foram observadas inconsistências taxonômicas ao incluir sequências de DNA, publicadas anteriormente e atribuídas à espécie *B. tridactylus*, à filogenia molecular. Erros de identificação ou processos como introgressão poderiam ser a causa de tal incongruência. Independente da origem, as divergências observadas levaram a proposições falsas em relação à distribuição geográfica, filogenia e taxonomia de *B. variegatus* e *B. tridactylus*.

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Literature Cited

Aguiar, J. M. 2004. The 2004 edentate species assessment workshop. Species summaries and species discussion. Edentata 6:3–26.

Anderson, R. P., and C. O. Handley, Jr. 2001. A new species of three-toed sloth (Mammalia: Xenarthra) from Panama, with a review of the genus *Bradypus*. Proceedings of the Biological Society of Washington 114:1–33.

Arnason, U., A. Gullberg, and A. Janke. 1997. Phylogenetic analyses of mitochondrial DNA suggest a sister group relationship between Xenarthra (Edentata) and Ferungulates. Molecular Biology and Evolution 14:762–768.

Ávila-Pires, F. D., and E. Gouveia. 1977. Mamíferos do Parque Nacional do Itatiaia. Boletim do Museu Nacional, Rio de Janeiro, Zoologia 291:1–29.

Avise, J. C. 2004. Molecular markers, natural history, and evolution. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

Barros, M. C., I. Sampaio, and H. Schneider. 2003. Phylogenetic analysis of 16S mitochondrial DNA data in sloths and anteaters. Genetics and Molecular Biology 26:5–11.

Barros, M. C., I. Sampaio, and H. Schneider. 2008. Novel 12S mtDNA findings in sloths (Pilosa, Folivora) and anteaters (Pilosa, Vermilingua) suggest a true case of long branch attraction. Genetics and Molecular Biology 31:793–799.

De Blainville, M.H.M.D. 1840. Osteógraphie ou description iconographique comparée du squelette et du système dentaire des cinq classes d'animaux vertébrés récents et fossiles pour servir de base a la zoologie et a la géologie. Mammifères.—Paresseaux.—G. Bradypus. Part 4. Arthus Bertrand, Paris, France.

Delsuc, F., F. M. Catzeflis, M. J. Stanhope, and E. J. P. Douzery. 2001. The evolution of armadillos, anteaters and sloths depicted by nuclear and mitochondrial phylogenies: implications for the status of the enigmatic fossil *Eurotamandua*. Proceedings of the Royal Society of London, B. Biological Sciences 268:1605–1615.

Delsuc, F., et al. 2002. Molecular phylogeny of living xenarthrans and the impact of character and taxon sampling on the placental tree rooting. Molecular Biology and Evolution 19:1656–1671.

Delsuc, F., S. F. Vizcaino, and E. J. Douzery. 2004. Influence of Tertiary paleoenvironmental changes on the diversification of South American mammals: a relaxed molecular clock study within xenarthrans. BMC Evolutionary Biology 4:11.

Eizirik, E., W. J. Murphy, and S. J. O'Brien. 2001. Molecular dating and biogeography of the early placental mammal radiation. Journal of Heredity 92:212–219.

Gannon, W. L., R. S. Sikes, and the Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809–823.

Gardner, A. L. (ed.). 2007 [dated 2007 but published 2008]. Mammals of South America. Vol. 1. Marsupials, xenarthrans, shrews, and bats. University of Chicago Press, Chicago, Illinois.

Gilmore, D. P., C. P. Costa, and D. P. F. Duarte. 2000. An update on the physiology of two- and three-toed sloths. Brazilian Journal of Medical Biology Research 33:129–146.

Good, J. M., et al. 2008. Ancient hybridization and mitochondrial capture between two species of chipmunks. Molecular Ecology 17:1313–1327.

Greenwood, A. D., J. Castresana, G. Feldmaier-Fuchs, and S. Pääbo. 2001. A molecular phylogeny of two extinct sloths. Molecular Phylogenetics and Evolution 18:94–103.

Hawksworth, D. L. 2004. Biological papers without cited voucher material are so much waste paper. European Journal of Science Education 30:81–83.

Hayssen, V. 2009. *Bradypus tridactylus* (Pilosa: Bradypodidae). Mammalian Species 839:1–9.

Hayssen, V. 2010. *Bradypus variegatus* (Pilosa: Bradypodidae). Mammalian Species 42(850):19–32.

International Union for the Conservation of Nature and Natural Resources (IUCN). 2009. IUCN Red list of threatened species. Version 2009.2. www.iucnredlist.org. Accessed 20 February 2010.

Lara-Ruiz, P., A. G. Chiarello, and F. R. Santos. 2008. Extreme population divergence and conservation implications for the rare endangered Atlantic Forest sloth, *Bradypus torquatus* (Pilosa: Bradypodidae). Biological Conservation 141:1332–1342.

McLenachan, P., and D. Penny. 2005. NCBI Genome Project. Massey University, Allan Wilson Centre for Molecular Ecology and Evolution, Palmerston North, New Zealand.

Moraes-Barros, N., C. Y. Miyaki, and J. S. Morgante. 2007. Identifying Management Units in non-endangered species: the example of the sloth *Bradypus variegatus* Schinz, 1825. Brazilian Journal of Biology 6:829–837.

Moraes-Barros, N., and J. S. Morgante. 2007. A simple protocol for the extraction and sequence analysis of DNA from study skin of museum collections. Genetics and Molecular Biology 30:1181–1185.

Moraes-Barros, N., J. A. B. Silva, C. Y. Miyaki, and J. S. Morgante. 2006. Comparative phylogeography of the Atlantic Forest endemic sloth (*Bradypus torquatus*) and the widespread three-toed sloth (*Bradypus variegatus*) (Bradypodidae, Xenarthra). Genetica 126:189–198.

MURPHY, W. J., T. H. PRINGLE, T. A. CRIDER, M. S. SPRINGER, AND W. MILLER. 2007. Using genomic data to unravel the root of the placental mammal phylogeny. Genetics Research 17:413–421.

NIKOLAEV, S., J. I. MONTOYA-BURGOS, E. H. MARGULIES, J. ROUGEMONT, B. NYFFELER, AND S. E. ANTONARAKIS. 2007. Early history of mammals is elucidated with the ENCODE multiple species sequencing data. PLoS Genetics 3:e2.

PAYNE, R. B., AND M. D. SORENSON. 2003. Museum collections as sources of genetic data. Bonner Zoologische Beiträge 51:97–104.

POINAR, H., M. KUCH, G. MCDONALD, P. MARTIN, AND S. PAABO. 2003. Nuclear gene sequences from a late Pleistocene sloth coprolite. Current Biology 13:1150–1152.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

PRASAD, A. B., M. W. ALLARD, AND E. D. GREEN. 2008. Confirming the phylogeny of mammals by use of large comparative sequence data sets. Molecular Biology and Evolution 25:1795–1808.

RANWEZ, V., F. DELSUC, S. RANWEZ, K. BELKHIR, M. K. TILAK, AND E. J. DOUZERY. 2007. OrthoMaM: a database of orthologous genomic markers for placental mammal phylogenetics. BMC Evolutionary Biology 7:241.

RODRÍGUEZ, F. J., J. L. OLIVER, A. MARÍN, AND J. R. MEDINA. 1990. The general stochastic model of nucleotide substitution. Journal of Theoretical Biology 142:485–501.

SANDERSON, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Molecular Biology and Evolution 19:101–109.

SANDERSON, M. J. 2003. R8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19:301–302.

SCHINZ, H. R. 1825. Das Thierreich eingetheilr nach dem Bau der Thiere als Grundlage ihrer Naturgeschihte und der vergleichenden Anatomie. J. G. Cotta, Stuttgart and Tübingen, Germany. Vol. 4.

SORENSON, M. D., AND R. B. PAYNE. 2001. A single origin of brood parasitism in African finches: implications for host–parasite coevolution. Evolution 55:2550–2567.

SORENSON, M. D., AND R. B. PAYNE. 2002. Molecular genetic perspectives on avian brood parasitism. Integrative and Comparative Biology 42:388–400.

STANHOPE, M. J., ET AL. 1998. Molecular evidence for multiple origins of Insectivora and for a new order of endemic African insectivore mammals. Proceedings of the National Academy of Sciences 95:9967–9972.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

TAMURA, K., AND M. NEI. 1993. Estimation of the number of nucleotide substitution in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512–526.

THOMAS, G. H. 2008. Phylogenetic distributions of British birds of conservation concern. Proceedings of the Royal Society of London, B. Biological Sciences 275:2077–2083.

TROUESSART, E. L. 1898. Catalogus mammalium tam viventium quam fossilium. Fasciculus V. Sirenia, Cetacea, Edentata, Marsupialia, Allotheria, Monotremata. R. Friedländer & Sohn, Berlin, Germany.

VIEIRA, C. D. C. 1955. Lista remissiva dos mamíferos do Brasil. Arquivos de Zoologia do Estado de São Paulo 8:341–474.

VILGALYS, R. 2003. Taxonomic misidentification in public DNA databases. New Phytologist 160:4–5.

WETZEL, R. M. 1982. Systematics, distribution, ecology, and conservation of South American edentates. Pp. 345–375 in Mammalian biology in South America (M. A. Mares and H. H. Genoways, eds.). Pymantuning Laboratory of Ecology, University of Pittsburgh, Pittsburgh, Pennsylvania.

WETZEL, R. M., AND F. D. ÁVILA-PIRES. 1980. Identification and distribution of the recent sloths of Brazil (Edentata). Revista Brasileira de Biologia 40:831–836.

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Appendix I

Detailed description of analyzed specimens presented by geographic locality. Taxonomic identification, as listed in museum collections, is presented along with the identification reviewed by morphological analysis of the skull (M). Species names in boldface type denote corrected taxonomic identification. DNA samples were obtained from some museum specimens and from specimens captured and sampled in the wild. Museum and DNA collection acronyms are defined in ''Materials and Methods.''

		Specimen locality	Specimen	Initial identification	Reviewed identification	Analysis (M, DNA)
Bolivia	Beni	Rio Mamoré—10°23′S, 65°23′W	AMNH209940	*B. variegatus*	*B. variegatus*	M
	Santa Cruz	Buena Vista—17°27′S, 63°40′W	AMNH61792	*B. variegatus*	*B. variegatus*	M
			FMNH51871	*B. variegatus*	*B. variegatus*	M
			FMNH21393	*B. variegatus*	*B. variegatus*	M
			FMNH21394	*B. variegatus*	*B. variegatus*	M
		Rio Suruto—17°24′S, 63°51′W	AMNH61791	*B. variegatus*	*B. variegatus*	M
		Santa Cruz de La Sierra—17°48′S, 63°10′W	AMNH133435	*B. variegatus*	*B. variegatus*	M
		Santa Cruz da La Sierra, 5 km E Rio Palometillas	AMNH 261304	*B. variegatus*	*B. variegatus*	M
Brazil	Alagoas	Manimbu—10°10′S, 36°22′W	MZUSP7528	*B. variegatus*	*B. variegatus*	M
		São Miguel dos Campos—09°46′S, 36°05′W	MZUSP7370	*B. variegatus*	*B. variegatus*	M
	Amapá	Estrada Campo Verde km 7, Porto Platou	MN20578	*B. tridactylus*	*B. tridactylus*	M
		RDS Rio Iratapuru, Lago Baliza	IEPA626	*B. tridactylus*	*B. tridactylus*	M, DNA
			IEPA627	*B. tridactylus*	*B. tridactylus*	M, DNA
		Cachoeira de Santo Antônio, Rio Jari, Ilha do Cemitério	MPEG21809	*B. tridactylus*	*B. tridactylus*	M
	Amazonas	Balbina—01°50′S, 59°30′W	MZUSP23159[a]	*B. tridactylus*	*B. tridactylus*	DNA
		Estirão do Equador, Rio Javari	MPEG1840	*B. variegatus*	*B. variegatus*	M
		Lago do Batista—03°18′S, 58°15′W	MZUSP5303	***B. tridactylus***	***B. variegatus***	M
			MN6064	*B. variegatus*	*B. variegatus*	M
			MN6065	*B. variegatus*	*B. variegatus*	M
		Maraã—1°48′S, 65°22′W	MZUSP13506	*B. variegatus*	*B. variegatus*	M
		Maraã—Setor Coraci, Rio Coracizinho, RDS Amanã	MPEG36645	*B. variegatus*	*B. variegatus*	M
		Rio Amazonas, south bank, Vila Bela Imperatriz, Santa Clara—2°36′S, 56°44′W	AMNH93108	***B. tridactylus***	***B. variegatus***	M
			AMNH93109	***B. tridactylus***	***B. variegatus***	M
			AMNH93110	***B. tridactylus***	***B. variegatus***	M
			AMNH93111	***B. tridactylus***	***B. variegatus***	M
			AMNH93112	***B. tridactylus***	***B. variegatus***	M
			AMNH93113	***B. tridactylus***	***B. variegatus***	M
			AMNH93114	***B. tridactylus***	***B. variegatus***	M
			AMNH93115	***B. tridactylus***	***B. variegatus***	M
			AMNH93104	***B. tridactylus***	***B. variegatus***	M
			AMNH93105	***B. tridactylus***	***B. variegatus***	M
			AMNH93106	***B. tridactylus***	***B. variegatus***	M
		Rio Amazonas, Santo Antonio do Amatary—03°00′S, 58°00′W	AMNHA93103	***B. tridactylus***	***B. variegatus***	M
		Rio Madeira, Rosarinho—34°02′S, 59°08′W	AMNH92845	*Bradypus sp.*	*B. variegatus*	M
			AMNH92829	***B. tridactylus***	***B. variegatus***	M
			AMNH92335	***B. tridactylus***	***B. variegatus***	M
		Rio Madeira, Rosarinho, Santo Antonio do Uayara	AMNH92333	***B. tridactylus***	***B. variegatus***	M
			AMNH92334	***B. tridactylus***	***B. variegatus***	M
			AMNH92332	***B. tridactylus***	***B. variegatus***	M
		Rio Negro—no specific locality	MN30471	***B. variegatus***	***B. tridactylus***	M
			MN30472	*B. variegatus*	*B. variegatus*	M
			MN30480	***B. variegatus***	***B. tridactylus***	M
			MN30482	*B. variegatus*	*B. variegatus*	M
		Rio Negro, Cacao Pereira—3°08′S, 60°05′W	AMNH80447	***B. tridactylus***	***B. variegatus***	M
			AMNH80448	***B. tridactylus***	***B. variegatus***	M
		Rio Negro, Manaus—3°08′S, 60°01′W	AMNH91353	***B. tridactylus***	***B. variegatus***	M
		Rio Negro, Manaus, Hacienda Rio Negro	AMNH78968	*B. tridactylus*	*B. tridactylus*	M
		Rio Solimoes, no specific locality	AMNH37155	***B. tridactylus***	***B. variegatus***	M
	Bahia	Bahia, Ilhéus—Atlantic Forest—14°47′S, 39°03′W	LABECBA1001[a]	*B. torquatus*	*B. torquatus*	DNA
			LABEC01014[a]	*B. torquatus*	*B. torquatus*	DNA
			LABECBA2006[a]	*B. variegatus*	*B. variegatus*	DNA
	Espírito Santo	Lagoa Juparana—19°20′S, 40°04′W	AMNH78844	***B. tridactylus***	***B. variegatus***	M
		no specific locality	MN23889	*B. variegatus*	*B. variegatus*	M
	Maranhão	Imperatriz	MPEG2440	*B. variegatus*	*B. variegatus*	M

APPENDIX I.—Continued.

	Specimen locality	Specimen	Initial identification	Reviewed identification	Analysis (M, DNA)
	Miritiba—2°36′S, 50°43′W	MZUSP2897	*B. variegatus*	*B. variegatus*	M
		MZUSP2898	*B. variegatus*	*B. variegatus*	M
		MZUSP2597	*B. variegatus*	*B. variegatus*	M
Minas Gerais	Passos—20°43′S, 46°37′W	MN23905	*B. variegatus*	*B. variegatus*	M
		MN23906	*B. variegatus*	*B. variegatus*	M
		MN23904	*B. variegatus*	*B. variegatus*	M
	Rio Novo	MN23888	*B. variegatus*	*B. variegatus*	M
	Teófilo Otoni—Atlantic Forest	LABEC02079[a]	*B. variegatus*	*B. variegatus*	DNA
Pará	Abaeté	MN2379	*B. variegatus*	*B. variegatus*	M
	Altamira	LABEC-Bvar47[a]	*B. variegatus*	*B. variegatus*	DNA
	Altamira, 85 km SW, E bank Rio Iriri—3°50′S, 52°40′W	USNM549523	*B. variegatus*	*B. variegatus*	M
	Belém, Utinga—1°27′S, 48°29′W	USNM339632	*B. variegatus*	*B. variegatus*	M
		MPEG2357	*B. variegatus*	*B. variegatus*	M
	Belém, Castanhosinho, Igarapé	MPEG1514	*B. variegatus*	*B. variegatus*	M
	Belém, Instituto Agronômico do Norte	MPEG2666	*B. variegatus*	*B. variegatus*	M
	Belém	MPEG946	***B. tridactylus***	***B. variegatus***	M
		MPEG969	***B. tridactylus***	***B. variegatus***	M
	Belterra—02°38′S, 54°57′W	MN5636	*B. variegatus*	*B. variegatus*	M
		MN5693	*B. variegatus*	*B. variegatus*	M
		MN5695	*B. variegatus*	*B. variegatus*	M
		MN5752	*B. variegatus*	*B. variegatus*	M
		MN5755	*B. variegatus*	*B. variegatus*	M
		MN5779	*B. variegatus*	*B. variegatus*	M
		MN5787	*B. variegatus*	*B. variegatus*	M
		MN5630	*B. variegatus*	*B. variegatus*	M
		MPEG22512	*B. variegatus*	*B. variegatus*	M
		MPEG20174	*B. variegatus*	*B. variegatus*	M
		MPEG20194	*B. variegatus*	*B. variegatus*	M
		MPEG20171	*B. variegatus*	*B. variegatus*	M
		MPEG20170	*B. variegatus*	*B. variegatus*	M
		MPEG20173	*B. variegatus*	*B. variegatus*	M
		MPEG20169	*B. variegatus*	*B. variegatus*	M
	Benevides, Retiro de Nazaré, Estância	MPEG2693	*B. variegatus*	*B. variegatus*	M
	Bravo	MZUSP5302	***B. tridactylus***	***B. variegatus***	M
	Cacoal	MPEG4649	*B. variegatus*	*B. variegatus*	M
	Cametá—02°15′S, 49°30′W	MZUSP5429	*B. variegatus*	*B. variegatus*	M
	Castanhal	MPEG2352	*B. variegatus*	*B. variegatus*	M
		MPEG2356	*B. variegatus*	*B. variegatus*	M
	Faro—02°11′S, 56°44′W	MN2383	*B. tridactylus*	*B. tridactylus*	M
	Fordlandia—3°40′S, 55°30′W	MZUSP13498	*B. variegatus*	*B. variegatus*	M
		MZUSP13501	*B. variegatus*	*B. variegatus*	M
		FMNH94551	*B. variegatus*	*B. variegatus*	M
		MZUSP13502	*B. variegatus*	*B. variegatus*	M
		MZUSP13497	*B. variegatus*	*B. variegatus*	M
	Igarapé Açu	MPEG2354	*B. variegatus*	*B. variegatus*	M
	Igarapé Açu, Lazarópolis do Prata	MPEG2353	***B. tridactylus***	***B. variegatus***	M
	Ilha de Marajó	FMNH34401	*B. variegatus*	*B. variegatus*	M
	Ilha de Marajó, Curralinho—10°00′S, 49°30′W	AMNH133438	***B. tridactylus***	***B. variegatus***	M
		MN23897	*B. variegatus*	*B. variegatus*	M
		MN5033	*B. variegatus*	*B. variegatus*	M
		MN5014	*B. variegatus*	*B. variegatus*	M
		AMNH133406	***B. tridactylus***	***B. variegatus***	M
		AMNH133419	***B. tridactylus***	***B. variegatus***	M
		AMNH133426	***B. tridactylus***	***B. variegatus***	M
		AMNH133432	***B. tridactylus***	***B. variegatus***	M
		AMNH133433	***B. tridactylus***	***B. variegatus***	M
		AMNH133455	***B. tridactylus***	***B. variegatus***	M
		MN5015	*B. variegatus*	*B. variegatus*	M
		MN5017	*B. variegatus*	*B. variegatus*	M
		MN5019	*B. variegatus*	*B. variegatus*	M
		MN5033	*B. variegatus*	*B. variegatus*	M
		MN5034	*B. variegatus*	*B. variegatus*	M

Appendix I.—Continued.

Specimen locality	Specimen	Initial identification	Reviewed identification	Analysis (M, DNA)
	MN5037	*B. variegatus*	*B. variegatus*	M
	MN5038	*B. variegatus*	*B. variegatus*	M
	MN5040	*B. variegatus*	*B. variegatus*	M
	MN5045	*B. variegatus*	*B. variegatus*	M
Ipixuna, Rio Capim Grande	MPEG23920	*B. variegatus*	*B. variegatus*	M
Juruti	MPEG38375	***B. tridactylus***	***B. variegatus***	M
No specific locality	FMNH25316	*B. variegatus*	*B. variegatus*	M
	FMNH25317	*B. variegatus*	*B. variegatus*	M
	FMNH25318	*B. variegatus*	*B. variegatus*	M
	FMNH25319	*B. variegatus*	*B. variegatus*	M
	MPEG6751	*B. variegatus*	*B. variegatus*	M
	MPEG6752	*B. variegatus*	*B. variegatus*	M
	MPEG6750	***B. tridactylus***	***B. variegatus***	M
	MPEG945	***B. tridactylus***	***B. variegatus***	M
	MPEG1475	*B. variegatus*	*B. variegatus*	M
	MPEG929	***B. tridactylus***	***B. variegatus***	M
	MPEG6749	*B. variegatus*	*B. variegatus*	M
Óbidos—01°55′S, 55°31′W	MN5962	*B. variegatus*	*B. variegatus*	M
Paragominas, Faz. Cauxi	MPEG26312	*B. variegatus*	*B. variegatus*	M
Parauapebas—East Amazon Forest	LABEC-AC109[a]	*B. variegatus*	*B. variegatus*	DNA
Patagonia, km 27	AMNH75140	***B. tridactylus***	***B. variegatus***	M
Porto Santarém	FMNH21551	*B. variegatus*	*B. variegatus*	M
Rio Majary, Recreio—01°42′S, 52°12′W	AMNH95841	***B. tridactylus***	***B. variegatus***	M
Rio Tapajós, Aramanay—02°45′S, 54°59′W	AMNH95102	***B. tridactylus***	***B. variegatus***	M
	AMNH95101	***B. tridactylus***	***B. variegatus***	M
Rio Tapajós, Igarape Amorin—02°26′S, 55°00′W	AMNH95329	***B. tridactylus***	***B. variegatus***	M
	AMNH95329	***B. tridactylus***	***B. variegatus***	M
Rio Tapajós, Inajatuba	AMNH95326	***B. tridactylus***	***B. variegatus***	M
	AMNH95327	***B. tridactylus***	***B. variegatus***	M
	AMNH95328	***B. tridactylus***	***B. variegatus***	M
	AMNH95325	***B. tridactylus***	***B. variegatus***	M
	AMNH95103	***B. tridactylus***	***B. variegatus***	M
Rio Tapajós, Caxiricatuba—02°50′S, 55°08′W	AMNH95104	***B. tridactylus***	***B. variegatus***	M
Rio Tapajós, Igarape Bravo—02°26′S, 55°00′W	AMNH95106	***B. tridactylus***	***B. variegatus***	M
	AMNH95105	***B. tridactylus***	***B. variegatus***	M
Rio Tocantins, Baiao—02°41′S, 49°41′W	AMNH96255	***B. tridactylus***	***B. variegatus***	M
Rio Tocantins, Ilha do Taiuna—02°15′S, 49°30′W	AMNH97315	***B. tridactylus***	***B. variegatus***	M
	AMNH96245	***B. tridactylus***	***B. variegatus***	M
	AMNH96246	***B. tridactylus***	***B. variegatus***	M
	AMNH96249	***B. tridactylus***	***B. variegatus***	M
	AMNH96250	***B. tridactylus***	***B. variegatus***	M
	AMNH96252	***B. tridactylus***	***B. variegatus***	M
	AMNH96242	***B. tridactylus***	***B. variegatus***	M
	AMNH97315	***B. tridactylus***	***B. variegatus***	M
	AMNH96244	***B. tridactylus***	***B. variegatus***	M
Rio Tocantins, Mocajuba—02°35′S, 49°30′W	AMNH96254	***B. tridactylus***	***B. variegatus***	M
	AMNH96253	***B. tridactylus***	***B. variegatus***	M
Rio Tocantins, Tucuruí, Ilha Tocantins	MPEG12479	*B. variegatus*	*B. variegatus*	M
Rio Tocantins, Tucuruí, Vila Brabo	MPEG12480	*B. variegatus*	*B. variegatus*	M
Rod. Belém–Brasilia km 307	MPEG1742	*B. variegatus*	*B. variegatus*	M
Santarém—north-central Brazil—02°26′S, 54°42′W	AMNH40830	***B. tridactylus***	***B. variegatus***	M
	MN23899	*B. variegatus*	*B. variegatus*	M
	MN23900	*B. variegatus*	*B. variegatus*	M
	MN23901	*B. variegatus*	*B. variegatus*	M
	MN23902	*B. variegatus*	*B. variegatus*	M
	USNM111636	*B. variegatus*	*B. variegatus*	M
	USNM239454	*B. variegatus*	*B. variegatus*	M
	MN23902	*B. variegatus*	*B. variegatus*	M
	AMNH40829	***B. tridactylus***	***B. variegatus***	M
	MPEG10232	*B. variegatus*	*B. variegatus*	M
	MPEG10233	*B. variegatus*	*B. variegatus*	M
	MPEG10235	*B. variegatus*	*B. variegatus*	M
	MPEG10236	*B. variegatus*	*B. variegatus*	M, DNA

APPENDIX I.—Continued.

	Specimen locality	Specimen	Initial identification	Reviewed identification	Analysis (M, DNA)
		MPEG10239	*B. variegatus*	*B. variegatus*	M
		MPEG20199	*B. variegatus*	*B. variegatus*	M
		MPEG20203	*B. variegatus*	*B. variegatus*	M
	Santarém, Ipanema	MN11596	*B. variegatus*	*B. variegatus*	M
		MN11597	*B. variegatus*	*B. variegatus*	M
	Santarém, Mojuí dos Campos—2°26′S, 54°42′W	USNM545912	*B. variegatus*	*B. variegatus*	M
		USNM545913	*B. variegatus*	*B. variegatus*	M
		USNM545914	*B. variegatus*	*B. variegatus*	M
		USNM545915	*B. variegatus*	*B. variegatus*	M
		USNM545916	*B. variegatus*	*B. variegatus*	M
		USNM545918	*B. variegatus*	*B. variegatus*	M
		USNM545919	*B. variegatus*	*B. variegatus*	M
		USNM545920	*B. variegatus*	*B. variegatus*	M
		USNM545921	*B. variegatus*	*B. variegatus*	M
		USNM545922	*B. variegatus*	*B. variegatus*	M
		USNM545924	*B. variegatus*	*B. variegatus*	M
		USNM545925	*B. variegatus*	*B. variegatus*	M
		USNM545926	*B. variegatus*	*B. variegatus*	M
		USNM545930	*B. variegatus*	*B. variegatus*	M
		USNM545931	*B. variegatus*	*B. variegatus*	M
		USNM545932	*B. variegatus*	*B. variegatus*	M
		USNM546934	*B. variegatus*	*B. variegatus*	M
		USNM545935	*B. variegatus*	*B. variegatus*	M
		USNM545936	*B. variegatus*	*B. variegatus*	M
		USNM545937	*B. variegatus*	*B. variegatus*	M
		USNM545911	*B. variegatus*	*B. variegatus*	M
		MPEG13282	*B. variegatus*	*B. variegatus*	M
		MPEG13269	*B. variegatus*	*B. variegatus*	M
		MPEG13283	*B. variegatus*	*B. variegatus*	M
		MPEG13285	*B. variegatus*	*B. variegatus*	M
		MPEG13287	*B. variegatus*	*B. variegatus*	M
		MPEG13263	*B. variegatus*	*B. variegatus*	M
		MPEG13265	*B. variegatus*	*B. variegatus*	M
		MPEG13274	*B. variegatus*	*B. variegatus*	M
		MPEG20192	*B. variegatus*	*B. variegatus*	M
		MPEG13271	*B. variegatus*	*B. variegatus*	M
		MPEG13272	*B. variegatus*	*B. variegatus*	M
		MPEG13278	*B. variegatus*	*B. variegatus*	M
		MPEG13270	*B. variegatus*	*B. variegatus*	M
		MPEG13277	*B. variegatus*	*B. variegatus*	M
		MPEG20193	*B. variegatus*	*B. variegatus*	M
		MPEG13275	*B. variegatus*	*B. variegatus*	M
		MPEG13273	*B. variegatus*	*B. variegatus*	M
		MPEG13262	*B. variegatus*	*B. variegatus*	M
		MPEG13264	*B. variegatus*	*B. variegatus*	M
		MPEG13281	*B. variegatus*	*B. variegatus*	M
		MPEG13268	*B. variegatus*	*B. variegatus*	M
		MPEG13276	*B. variegatus*	*B. variegatus*	M
	Santarém, Santarém–Cuiabá km 35	USNM461731	*B. variegatus*	*B. variegatus*	M
	Santarém, Santarém–Cuiabá km 16	MPEG8533	*B. variegatus*	*B. variegatus*	M
	Taperinha	MPEG4648	***B. tridactylus***	***B. variegatus***	M
		MPEG4650	*B. variegatus*	*B. variegatus*	M
	Vigia, São Francisco	MPEG2358	*B. variegatus*	*B. variegatus*	M
Rio de Janeiro	Barreiros	MN1162	*B. variegatus*	*B. variegatus*	M
	Rio de Janeiro, Jacarepaguá	AMNH133437	***B. tridactylus***	***B. variegatus***	M
	Parati, Pedra Branca—23°13′S, 44°43′W	MN6103	*B. variegatus*	*B. variegatus*	M
		MN6702	*B. variegatus*	*B. variegatus*	M
		MN7608	*B. variegatus*	*B. variegatus*	M
		MN7609	*B. variegatus*	*B. variegatus*	M
		MN8450	*B. variegatus*	*B. variegatus*	M
		MN5650	*B. variegatus*	*B. variegatus*	M
	Teresópolis—02°26′S, 45°09′W	MN2387	*B. variegatus*	*B. variegatus*	M
	Teresópolis, Fazenda Boa Fé	MN7615	*B. variegatus*	*B. variegatus*	M

Appendix I.—Continued.

		Specimen locality	Specimen	Initial identification	Reviewed identification	Analysis (M, DNA)
			MN23892	*B. variegatus*	*B. variegatus*	M
			MN7262	*B. variegatus*	*B. variegatus*	M
	Roraima	Rio Mucajai, Rio Branco—Northwest Amazon Forest—2°22′N 60°58′W	MZUSP13500	*B. variegatus*	*B. variegatus*	M, DNA
	São Paulo	Santos	USNM63004	*B. variegatus*	*B. variegatus*	M
		São Paulo, Jaraguá—23°27′S, 46°44′W	FMNH94296	*B. variegatus*	*B. variegatus*	M
		São Paulo—Atlantic Forest	LABECDpv13431[a]	*B. variegatus*	*B. variegatus*	DNA
			LABEC02050[a]	*B. variegatus*	*B. variegatus*	DNA
Colombia		No specific locality	FMNH88489	*B. variegatus*	*B. variegatus*	M
	Bolívar	San Juan Nepomuceno—09°58′N, 75°04′W	FMNH68916	*B. variegatus*	*B. variegatus*	M
	Caquetá	No specific locality	FMNH140254	***B. tridactylus***	***B. variegatus***	M
	Cauca	Rio Saija—02°52′N, 77°41′W	FMNH90060	*B. variegatus*	*B. variegatus*	M
	Cesar	Colombia: Colonia Agrícola de Caracolicito—10°18′N, 74°00′W	USNM281352	*B. variegatus*	*B. variegatus*	M
			USNM281353	*B. variegatus*	*B. variegatus*	M
	Choco	Golfo de Uraba, Unguia—08°01′N, 77°97′W	FMNH69587	*B. variegatus*	*B. variegatus*	M
			FMNH69588	*B. variegatus*	*B. variegatus*	M
			FMNH69589	*B. variegatus*	*B. variegatus*	M
			FMNH69590	*B. variegatus*	*B. variegatus*	M
	Córdoba	Catival, upper Rio San Jorge—08°17′N, 75°41′W	FMNH68921	*B. variegatus*	*B. variegatus*	M
			FMNH68919	*B. variegatus*	*B. variegatus*	M
			FMNH68920	*B. variegatus*	*B. variegatus*	M
		Rio Baudo, Rio Sando—05°03′N, 76°57′W	FMNH90061	*B. variegatus*	*B. variegatus*	M
	Putumayo	Rio Mecaya—02°8′N, 75°20′W	FMNH70812	*B. variegatus*	*B. variegatus*	M
			FMNH70813	*B. variegatus*	*B. variegatus*	M
	Sucre	Coloso, Las Campanas—09°30′N, 75°21′W	FMNH68918	*B. variegatus*	*B. variegatus*	M
	Valle del Cauca	Zabaletas, 500 m	FMNH86762	*B. variegatus*	*B. variegatus*	M
			FMNH86879	*B. variegatus*	*B. variegatus*	M
Costa Rica	Cartago	Angostura—09°53′N, 83°38′W	USNM12871	*B. variegatus*	*B. variegatus*	M
	Limón	Talamanca	USNM12103	*B. variegatus*	*B. variegatus*	M
Ecuador	Napo	Rio Suno—04°2′S, 77°08′W	FMNH31119	*B. variegatus*	*B. variegatus*	M
Guyana		No specific locality	AMNH130106	*B. tridactylus*	*B. tridactylus*	M
			AMNH140498	*B. tridactylus*	*B. tridactylus*	M
			FMNH16557	*B. tridactylus*	*B. tridactylus*	M
			FMNH16556	*B. tridactylus*	*B. tridactylus*	M
	Cuyuni-Mazaruni	Essequibo, Kartabo Point—06°23′N, 58°41′W	AMNH42454	*B. tridactylus*	*B. tridactylus*	M
			AMNH48180	*B. tridactylus*	*B. tridactylus*	M
			AMNH48369	*B. tridactylus*	*B. tridactylus*	M
			AMNH74131	*B. tridactylus*	*B. tridactylus*	M
			AMNH74137	*B. tridactylus*	*B. tridactylus*	M
	Upper Takutu–Upper Essequibo	Dadanawa—02°50′N, 59°30′W	USNM362241	*B. tridactylus*	*B. tridactylus*	M
Honduras		Gracias a Dios, Patuca River	USNM21011	*B. variegatus*	*B. variegatus*	M
Nicaragua		Escondido River—12°09′N, 83°46′W	USNM51273	*B. variegatus*	*B. variegatus*	M
		El Recreo, Atlantico Sur—12°09′N, 84°26′W	USNM337714	*B. variegatus*	*B. variegatus*	M
Panama	Bocas del Toro	Isla San Cristóbal, Bocatorito—09°15′N, 82°16′W	USNM449525	*B. variegatus*	*B. variegatus*	M
	Darién	Cerro Tacarcuna—08°10′N, 77°18′W	USNM338124	*B. variegatus*	*B. variegatus*	M
		El Real—08°06′N, 77°45′W	AMNH37621	*B. variegatus*	*B. variegatus*	M
	Colón	Gatun—09°15′N, 79°56′W	AMNH36816	*B. variegatus*	*B. variegatus*	M
	Panamá	Barro Colorado Island—09°09′N, 79°51′W	FMNH30738	*B. variegatus*	*B. variegatus*	M
		La Chorrera—08°52′N, 79°48′W	AMNH31427	*B. variegatus*	*B. variegatus*	M
Peru		No specific locality	AMNH98530	*B. variegatus*	*B. variegatus*	M
	Loreto	Alto Amazonas, Rio Morona, boca Rio Amaya—04°39′S, 77°07′W	FMNH88893	*B. variegatus*	*B. variegatus*	M
		Iquitos—03°46′S, 73°15′W	AMNH98545	*B. variegatus*	*B. variegatus*	M
			AMNH98546	*B. variegatus*	*B. variegatus*	M
			AMNH98533	*B. variegatus*	*B. variegatus*	M
		Nauta, Rio Samiria, Santa Helena—04°50′S, 74°13′W	FMNH86896	*B. variegatus*	*B. variegatus*	M
		Rio Amazonas, Apayacu—03°19′S, 72°06′W	AMNH74429	*B. variegatus*	*B. variegatus*	M
		Rio Amazonas, Orosa—03°26′S, 72°08′W	AMNH73758	*B. variegatus*	*B. variegatus*	M
			AMNH73759	*B. variegatus*	*B. variegatus*	M
		Rio Amazonas, Puerto Indiana—03°28′S, 73°03′W	AMNH73757	*B. variegatus*	*B. variegatus*	M

APPENDIX I.—Continued.

		Specimen locality	Specimen	Initial identification	Reviewed identification	Analysis (M, DNA)
			AMNH73572	*B. variegatus*	*B. variegatus*	M
			AMNH73573	*B. variegatus*	*B. variegatus*	M
		Rio Samiria—04°42′S, 74°13′W	AMNH188196	*B. variegatus*	*B. variegatus*	M
			AMNH76497	*B. variegatus*	*B. variegatus*	M
			AMNH76403	*B. variegatus*	*B. variegatus*	M
		Rio Ucayali, Sarayacu—06°44′S, 75°06′W	AMNH76495	*B. variegatus*	*B. variegatus*	M
			AMNH76496	*B. variegatus*	*B. variegatus*	M
			AMNH76408	*B. variegatus*	*B. variegatus*	M
		Yurimaguas, Puerto Arturo—05°50′S, 76°03′W	FMNH20132	*B. variegatus*	*B. variegatus*	M
South America		South America—Zoo	FMNH60164	*B. variegatus*	*B. variegatus*	M
Suriname	Paramaribo	Paramaribo, 900 feet—05°50′N, 55°10′W	FMNH93297	*B. tridactylus*	*B. tridactylus*	M
	Saramacca	La Poule	FMNH95444	*B. tridactylus*	*B. tridactylus*	M
Unknown		Unknown	MN1694	***B. tridactylus***	***B. variegatus***	M
Venezuela	Amazonas	Mount Duida, Esmeralda—03°10′N, 65°33′W—left bank Rio Orinoco	AMNH76904	***B. tridactylus***	***B. variegatus***	M
		Rio Casiquiare, left bank (translated), El Merey—03°05′N, 65°05′W	AMNH78515	***B. tridactylus***	***B. variegatus***	M
	Bolívar	Camarata Valley, 450 m	AMNH135474	***B. variegatus***	***B. tridactylus***	M
		Ciudad Bolívar—08°08′N, 63°33′W	AMNH16135	*B. tridactylus*	*B. tridactylus*	M
		El Manaco—06°17′N, 61°19′W—59 km SE El Dorado	USNM374821	*B. tridactylus*	*B. tridactylus*	M
		Los Patos—07°11′N, 62°22′W—25 km SE El Manteco	USNM374822	*B. tridactylus*	*B. tridactylus*	M
		Rio Suapure—06°48′N, 67°01′W	AMNH16933	*B. tridactylus*	*B. tridactylus*	M
			AMNH17560	*B. tridactylus*	*B. tridactylus*	M
	Miranda	San Andrés—10°22′N, 65°50′W—16 km SSE Caracas	USNM372832	*B. variegatus*	*B. variegatus*	M

[a] For specimens captured and sampled in the wild from which DNA samples were obtained, taxonomic identifications were made using external morphology.